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Progressive increase of $CD4^+/CD45RC^-$ lymphocytes after allograft rat lung transplantation: A marker of acute rejection

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Background: Acute rejection remains one the most serious problems in lung transplantation. Although biopsy has been used for assessing the dysfunction of grafts, it is difficult to determine rejection at an early stage. Lymphocyte infiltration and activation play an important role in acute rejection of transplanted organs, and the dynamic change of lymphocyte subpopulations might be a marker to determine graft rejection after lung transplantation.

Methods: A rat lung transplant model was used. Graft-infiltrating lymphocytes in lung tissues were examined by means of histology, and isolated cells were analyzed by means of flow cytometry. Phenotypes of lymphocytes in the regional and remote lymph nodes, spleen, peripheral blood, and bronchoalveolar lavage fluid were also measured by means of flow cytometry.

Results: After allograft transplantation, increased lymphocytes were seen in allografts but not in isografts. In allografts the percentage of T cells increased from day 1 to day 5, whereas that of B cells was decreased. The $CD4^+/CD8^+$ ratio decreased in allografts. The proportion of $CD4^+/CD45RC^-$ cells increased in the allografts, which was mainly due to the increase of $CD45RC^-$ cells in the total $CD4^+$ cells. Similar changes were found in regional mediastinal lymph nodes but not in the mesenteric lymph nodes, spleen, or peripheral blood. Thus this is a specific response to lung allografts. Importantly, $CD45RC^-$ cells were significantly increased in the bronchoalveolar lavage fluid.

Conclusion: Significant change of lymphocyte subpopulations is a sign of lymphocyte activation. Increased $CD4^+/CD45RC^-$ cells in lung allografts could be an early marker of acute rejection, which can be examined by means of lung lavage and flow cytometry.

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Lung transplantation is an effective clinical option for end-stage lung diseases. However, the incidence of acute rejection is higher after lung transplantation than after other solid organ transplantations.[1] Five percent of deaths during the first 30 days after lung transplantation are directly caused by acute rejection.[2] Although biopsy has been used for assessing the dysfunction of the grafts, it is difficult to determine the rejection at the early stage.

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Lymphocyte infiltration and activation play an important role in acute rejection of transplanted grafts. We have previously demonstrated that in a rat lung transplantation model lymphocyte infiltration had a latent phase on day 1, a vascular phase on day 3, and an alveolar phase on day 5, with the maximal infiltration on day 5 after transplantation.[3,4] To determine whether the increased lymphocyte infiltration in lung grafts is associated with changes of subpopulations, in the present study we examined the expression of T cells, B cells, and $CD4^+/CD8^+$ ratio. We focused on the expression of a subset of CD45 markers in $CD4^+$ cells.

CD45 is a transmembrane molecule expressed by all nucleated hematopoietic cells and their precursors.[5] A long intracytoplasmic tail of CD45 possesses 2 protein tyrosine phosphatase domains. It has been suggested that CD45 is a drug target and central regulator involved in differentiation of multiple hematopoietic cell lineages, autoimmunity, and antiviral immunity.[6] CD45 is expressed in several isoforms generated by means of alternative mRNA splicing of variable CD45 exons. By using specific monoclonal antibodies (mAbs), restricted regions have been identified from CD45 isoforms. They are expressed as CD45R, followed by a letter to indicate the specific region where the mAbs bind. For example, in rats the MRC-OX22 mAb binds to CD45RC. CD45 isoforms are divided into $CD45R^{high}$ (high-molecular-weight isoforms, 220-190 kd) and $CD45R^{low}$ (low-molecular-weight isoforms, 180 kd). The $CD45R^{low}$ cells are detected in human subjects on the basis of antibodies to CD45RO, such as UCHL-1. The $CD45R^{low}$ cells in mice and rats are identified by a lack of staining with the antibodies for CD45RB and CD45RC, respectively.[7]

$CD4^+$ T cells can be divided into subpopulations on the basis of the status of their CD45RC expression. These subpopulations of $CD4^+$ cells have different functions. By means of mitogenic stimulation, $CD4^+/CD45RC^+$ cells produced interleukin 2 and interferon γ and showed little expression of interleukin 4 mRNA, whereas $CD4^+/CD45RC^-$ cells showed the opposite result.[8] The shift from $CD45R^{high}$ ($CD45RA/B/C^+$) to $CD45R^{low}$ (CD45RO) in human subjects, from $CD45RB^+$ to $CD45RB^-$ in mice, and from $CD45RC^+$ to $CD45RC^-$ in rats has been observed under different conditions, including immunologic memory and activation.[9-12] In human subjects several reports have shown that after solid organ transplantation (kidney, heart, and liver), the severity of rejection is significantly associated with the change of infiltrating T cells from those expressing $CD45^{high}$ molecules (CD45RA) toward those expressing $CD45R^{low}$ molecules (CD45RO).[13-16] However, in most of these studies $CD45R^{high}$ or $CD45R^{low}$ cells are not double stained with $CD4^+$ marker.

The changes of CD45R isoforms in lung allografts are especially unclear; the subpopulations of $CD4^+$ cells in lung allografts according to their expression of CD45R markers have not been well studied. In the present study, using a rat lung transplantation model, we investigated the expression of CD45RC in $CD4^+$ T cells in the grafts and in the recipients (the regional and nonregional lymph nodes of the lung, the spleen, and the peripheral blood) with the progress of acute graft rejection. In addition, bronchoalveolar lavage fluid (BALF) collected from allografts was analyzed to determine whether the changes in the grafted lung tissues could be examined from the BALF. Our results showed that $CD4^+/CD45RC^-$ cells in the lung allografts could be an indicator for the advance of acute rejection.

Materials and Methods

Animals

Inbred male rats of the LEW (RTl^1) and BN (RTl^n) strains were obtained from Charles River (Kanagawa, Japan). Rats from 12 to 16 weeks old were used in all experiments. Animals were housed in pathogen-free conditions and received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised 1996, and institutional guidelines.

Transplantation

Left lungs from BN or LEW donors were orthotopically transplanted to LEW recipients according to the modified technique of Prop and colleagues.[17] Briefly, the recipient rat was anesthetized with oxygen, nitrous oxide, and halothane (4%) and intubated. The recipient rat was artificially ventilated at a rate of 90 breaths/min and a tidal volume of 7 mL/kg with oxygen, nitrous oxide, and halothane (1.5%). After a left-side thoracotomy, the native left lung and the postcaval lobe of the right lung were dissected. The donor rat was given pentobarbital (50 mg/kg) intraperitoneally and ventilated at a rate of 90 breaths/min and a tidal volume of 7 mL/kg. A donor left lung was resected after flushing through the pulmonary artery with cold normal saline. The donor lung was implanted into the recipient's chest. By using an operating microscope, the pulmonary vein and artery were anastomosed with a continuous suture (10-0 nylon, Dermalon; American Cyanamid Co, Danbury, Conn). The bronchial anastomosis was performed with an interrupted suture (10-0 nylon). After the initiation of ventilation and reperfusion, the thorax was closed, leaving a chest drainage tube. This tube was removed after the recipient rat recovered from anesthesia.[18]

Preparation of Lymphocytes from Grafts, Spleen, Lymph Nodes, and Peripheral Blood in Recipient Rats

Recipient rats were killed on days 1, 3, or 5 after the operation. The rats were given pentobarbital (50 mg/kg administered intraperitoneally) and ventilated artificially. One milliliter of peripheral blood was obtained by means of tail vein puncture with heparinized syringes. After laparotomy, the spleen was removed and saved in cold phosphate-buffered saline (PBS). After thoracotomy, the left atrium was incised to wash out blood in pulmonary vessels, and the grafted lung was irrigated with 100 mL of cold saline solution administered through the pulmonary arterial trunk with 20 cm H_2O

pressure. The heart and lungs were excised, and the left lung was removed. The mediastinal lymph nodes, which were the regional lymph nodes in the chest, and the mesenteric lymph nodes, which were the nonregional lymph nodes, were collected separately. The mediastinal lymph nodes in this study included the parathymic and the posterior mediastinal lymph nodes, which drain the thoracic viscera of the rat.[19]

Cells were segregated from lungs according to a method described by Holt and associates.[20] The lungs were agitated in a complete medium (RPMI 1640 with 10% fetal bovine serum [FBS] and 10^{-5} mol/L 2-mercaptoethanol) containing 10 mg/mL collagenase and 1 mg/mL DNase. To each sample was added 10 mL of RPMI 1640 medium, and the mixture was then incubated at 37°C for 90 minutes. Then cells were filtered through cotton and washed with RPMI 1640 medium twice. Cells were adjusted to a final concentration of 1.0×10^7/mL with the RPMI 1640 medium. Lymphocytes of the grafted lungs were separated with Lymphocyte-Rat (Cedarlane, Ontario, Canada) as a density separation medium by means of centrifugation (20 minutes, 400*g*, room temperature) and then washed 2 times with the medium. Cells were adjusted to the proper concentration in PBS containing 2% FBS and 0.02% sodium azide for phenotype analysis by means of flow cytometry.

Single-cell suspensions from the lymph nodes or spleen were prepared by teasing tissue through a nylon mesh screen, and then the cells were collected in PBS containing 2% FBS and 0.02% sodium azide. The red blood cells were lysed with Tris–ammonium chloride. The cells were adjusted to the proper concentration in PBS containing 2% FBS and 0.02% sodium azide for flow cytometry.

Bronchoalveolar Lavage for Allografted Lungs

Bronchoalveolar lavage was performed for a number of the allografted lungs. Two milliliters of BALF was recovered from each graft. An 18-gauge needle with its point removed was inserted into the left main bronchus of a graft, and it was then bound with thread. Cold PBS (0.5 mL) was injected into the bronchus each time until 2.0 mL of BALF was recovered.

Antibodies

Fluorescein isothiocyanate (FITC)–conjugated OX-22 (anti-CD45RC), FITC-conjugated OX-19 (anti-CD5), and FITC-conjugated OX-33 (anti-B cell) mAbs were purchased from Serotec (Oxford, United Kingdom). Biotin-conjugated W3/25 (anti-CD4) and FITC-conjugated OX-8 (anti-CD8) mAbs were kindly provided by Dr K. Himeno (Tokushima, Japan).

Flow Cytometry

For 2-color flow cytometry, cells from each sample were stained for 30 minutes with optimal dilutions of antibodies: biotin-conjugated W3/25 (anti-CD4) and FITC-conjugated OX-8 (anti-CD8) or biotin-conjugated W3/25 and FITC-conjugated OX-22 (anti-CD45RC). Each sample was incubated with avidin-phycoerythrin for 30 minutes. For single-color flow cytometry, cells from each sample were stained for 30 minutes with optimal dilutions of antibodies: FITC-conjugated OX-19 (anti-CD5) and FITC-conjugated OX-33 (anti-B cell). The labeled cells were analyzed with a FACScan (Becton-Dickinson, Mountain View, Calif). PBS containing 2% FBS and 0.02% sodium azide was used for all flow cytometric analyses. Dead cells and nonlymphoid cells were excluded from analysis by means of selective gating on the basis of anterior and right-angle scatter.

Graft-infiltrating Cells

The lymphoid cells isolated from the grafted lungs were analyzed by means of flow cytometry. The lymphocytes that were morphologically separated by means of anterior and right-angle scatter measurements were defined as graft-infiltrating lymphocytes (GILs). The accuracy of this separation was confirmed by means of double staining with biotin-conjugated W3/25 (anti-CD4) and FITC-conjugated OX-8 (anti-CD8) mAbs.

Statistics

All data are expressed as means ± SD. Data were analyzed by using the Bartlett test and 1-way analysis of variance, followed by the Scheffe method for multiple comparisons among groups. For comparison between isografts and allografts, the unpaired Student *t* test was applied.

Results

Lymphocyte Infiltration in Allograft Lungs

In the allografts peribronchial and perivascular edema was observed on day 1 after transplantation. On day 3, perivascular lymphocyte infiltration, predominantly in the peripheral pulmonary vein, was found. On day 5, lymphocytes formed peribronchial and perivascular cuffing and infiltrated into the interstitium, which was associated with distortion of lung structure (Figure 1). In contrast, although peribronchial and perivascular edema was observed on days 3 and 5 after transplantation, lymphocyte infiltrations were not seen in isografts (Figure 1).

Changes of GIL Profiles in Allografts

In the normal rat lung the number of lymphocytes in the lung is very low and difficult to analyze (data not shown). In isografts lymphocytes in the lung were mainly T cells (53% and 57% on days 3 and 5, respectively). B cells were about 20% of total infiltrating cells (Table 1). In the allografts the proportion of T cells, expressed as $CD5^+$ cells, increased from 33% to 54% from day 1 to day 5 after transplantation. Meanwhile, B cells decreased from 41% to 30% ($P < .05$, Table 1).

The expression of $CD4^+$ and $CD8^+$ cells in isografts was relatively stable from day 3 to day 5. In allografts, although $CD4^+$ cells increased from 35% to 45% from day 1 to day 5, the increase of $CD8^+$ cells was from 7% to 17%. Therefore the $CD4^+/CD8^+$ ratio decreased from 5.2% to 2.8% ($P < .05$, Table 1). The expression of T cells ($CD5^+$ cells) also increased in allografts but remained constant in the isografts. Taken together, in the allografts not only did the lymphocyte infiltration significantly increase but also the subpopulations of lymphocytes changed in a time-depen-

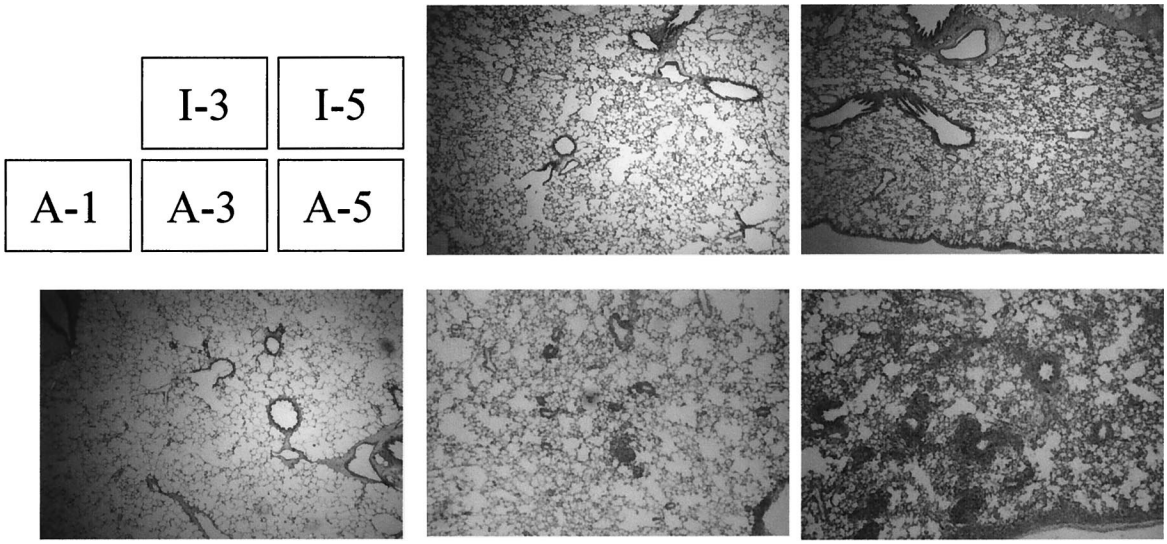

Figure 1. Lymphocyte infiltration in allograft lungs. A time-dependent increase in lymphocytes was seen in allografts from day 1 to day 5 after transplantation, which was not seen in isografts on days 3 and 5. (Hematoxylin and eosin staining; original magnification 40×.)

TABLE 1. Changes of GIL profiles after lung transplantation

	Allograft			Isograft	
	Day 1 (n = 3)	Day 3 (n = 7)	Day 5 (n = 6)	Day 3 (n = 3)	Day 5 (n = 3)
T cells ($CD5^+$), %	33.6 ± 3.3	47.9 ± 2.8	54.1 ± 5.6*	52.5 ± 3.7	56.9 ± 7.0
B cells, %	41.5 ± 4.7	21.5 ± 5.6†	30.0 ± 6.5*	24.3 ± 2.3	21.9 ± 4.1
$CD4^+$, %	35.5 ± 4.5	44.3 ± 6.8	44.7 ± 5.3‡	49.2 ± 4.9	54.0 ± 0.3
$CD8^+$, %	7.0 ± 1.5	12.7 ± 4.5	16.8 ± 4.4*	12.0 ± 2.9	18.4 ± 3.4
CD4/CD8 ratio	5.2 ± 1.4	3.8 ± 0.9	2.8 ± 0.7*	4.3 ± 1.1	3.0 ± 0.5

*$P < .05$ compared with day 1 by using Scheffe method.
†$P < .01$ compared with day 1 by using Scheffe method.
‡$P < .05$ compared with isografts on day 5 by using unpaired Student *t* test.

dent manner. These phenomena are allograft specific because they are not seen in isografts.

Proportion of $CD4^+/CD45RC^-$ Cells Increased During Acute Rejection

We then analyzed the expression of CD45RC in the GILs by means of dual staining of cells, followed by flow cytometry. Representative results are shown in Figure 2. The proportion of $CD4^+/CD45RC^-$ cells to GILs increased from 20% to 38% during the process of acute rejection in allografts (from day 1 to day 5) but not in the isografts between day 3 and day 5 after lung transplantation (Figure 3, *A*). The proportion of $CD4^+/CD45RC^-$ cells to GILs in allografts was also significantly higher than that in isografts on both days 3 and 5 (Figure 3, *A*).

Considering that the proportion of $CD4^+$ cells in GILs also increased during the progress of acute rejection (Table 1), we then focused on the proportion of $CD4^+/CD45RC^-$ cells in the $CD4^+$ cell population. The proportion of $CD4^+/CD45RC^-$ cells to $CD4^+$ cells in GILs increased with the advance of acute rejection and significantly increased from 57% on day 1 to 86% on day 5 ($P < .01$; Figure 3, *B*) after allotransplantation. In the isografted lungs the proportion of $CD4^+/CD45RC^-$ cells to $CD4^+$ cells in GILs did not change between days 3 and 5 (Figure 3, *B*). The proportion of $CD4^+/CD45RC^-$ in $CD4^+$ cells on days 3 and 5 in allografts was significantly higher than that in isografts on days 3 and 5, respectively. Therefore during allograft rejection, the increased $CD4^+$ cells contain more $CD45RC^-$ cells.

Changes of $CD4^+/CD45RC^-$ Cells in Other Lymphocytic Tissues

We also analyzed the proportion of $CD4^+/CD45RC^-$ cells to total lymphocytes and the proportion of $CD4^+/CD45RC^-$ cells to $CD4^+$ cells in mediastinal and mesen-

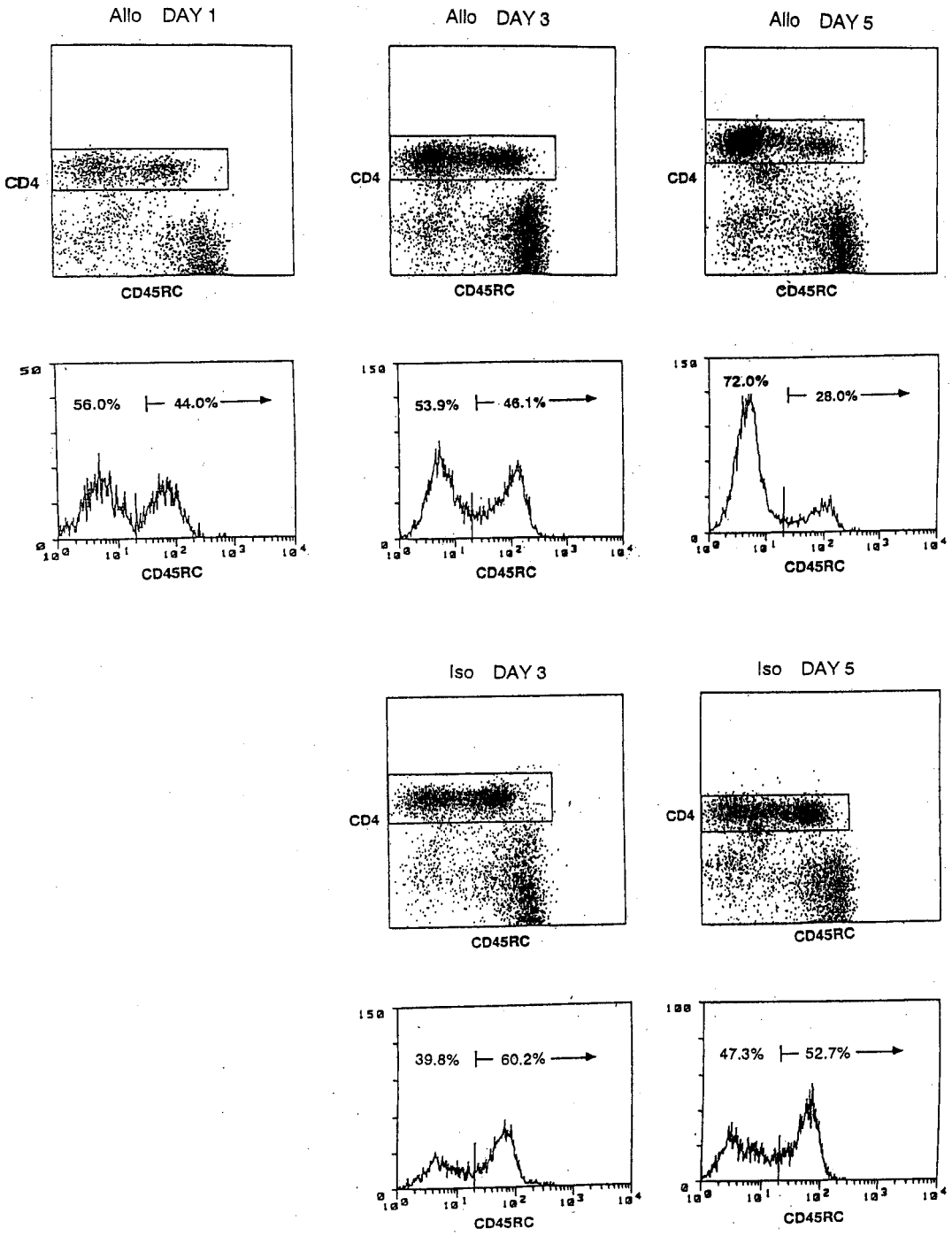

Figure 2. Representative results of the FACS analysis in the allografts (on days 1, 3, and 5) and in the isografts (on days 3 and 5) after lung transplantation. *Top,* Two-dimensional dot-plotting graphs show the results of the 3-parameter analysis by CD4 (biotin-conjugated W3/25 and avidin-phycoerythrin) and CD45RC (FITC-conjugated OX-22) staining for GILs. *Bottom,* Histograms show percentages of CD45RC and CD45RC$^-$ cells in CD4$^+$ cells.

teric lymph nodes, spleen, and peripheral blood lymphocytes (PBLs). In the mediastinal lymph nodes of the recipient rats, the proportion of CD4$^+$/CD45RC$^-$ cells to total lymphoid cells on day 5 was significantly increased over that on day 3 after allograft lung transplantation (Table 2). The proportion of CD4$^+$/CD45RC$^-$ cells to CD4$^+$ cells

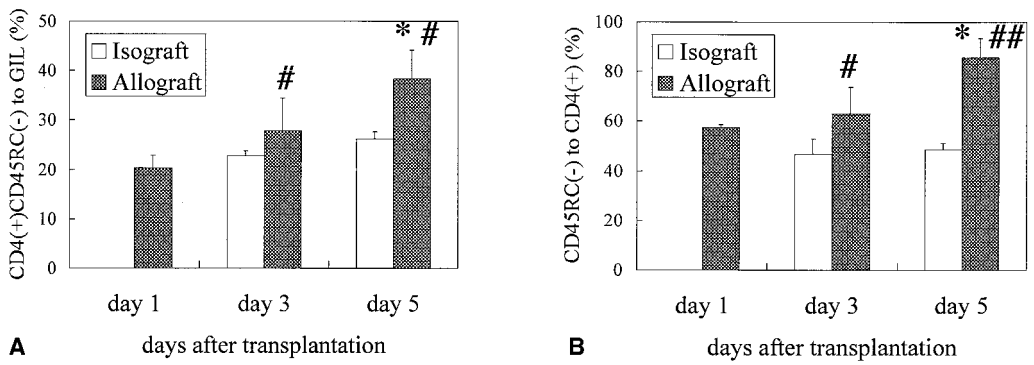

Figure 3. Significant increase of CD4+/CD45RC− cells in the lung allografts. A, The proportion of $CD4^+/CD45RC^-$ cells to GILs was increased during the progress of acute rejection in allografts. *$P < .05$ compared with that on days 1 and 3 in allografts by using the Scheffe method. #$P < .05$ compared with isografts by using the Student *t* test. B, Proportion of $CD4^+/CD45RC^-$ cells to $CD4^+$ cells in GILs was increased during acute rejection in allografts. *$P < .01$ compared with that on days 1 and 3 in allografts by using the Scheffe method; #$P < .05$ and ##$P < .01$ compared with isografts by using the Student *t* test.

TABLE 2. Proportion of $CD4^+/CD45RC^-$ cells in lymphocytes collected from lymph nodes, spleen, and PBLs

	Mediastinal LN (%)	Mesenteric LN (%)	Spleen (%)	PBL (%)
Allograft				
Day 1 (n = 3)	12.5 ± 1.2	15.9 ± 0.6	19.2 ± 1.2	13.4 ± 1.8
Day 3 (n = 7)	8.5 ± 2.5	16.5 ± 5.1	16.3 ± 3.8	18.9 ± 4.3
Day 5 (n = 6)	15.8 ± 3.8*	20.6 ± 3.3	20.6 ± 6.1	18.5 ± 2.7
Isograft				
Day 1 (ND)	—	—	—	—
Day 3 (n = 7)	10.1 ± 0.5	16.0 ± 1.0	18.3 ± 3.7	16.6 ± 1.3
Day 5 (n = 6)	11.5 ± 2.6	18.1 ± 3.5	16.7 ± 2.9	19.8 ± 1.9†

ND, Not done.
*$P < .01$ compared with day 3 in allografts by using Scheffe method.
†$P < .05$ compared with day 3 in isografts by using Scheffe method.

TABLE 3. Proportion of $CD45RC^-$ cells in $CD4^+$ cells in lymph nodes, spleen, and PBLs

	Mediastinal LN (%)	Mesenteric LN (%)	Spleen (%)	PBL (%)
Allograft				
Day 1 (n = 3)	29.2 ± 4.8	30.6 ± 1.7	42.9 ± 2.5	31.4 ± 1.2
Day 3 (n = 7)	31.6 ± 4.6	34.2 ± 6.2	38.3 ± 4.8	33.0 ± 5.8
Day 5 (n = 6)	43.9 ± 8.5*	40.8 ± 5.5	45.8 ± 7.6	35.9 ± 5.7
Isograft				
Day 1 (ND)	—	—	—	—
Day 3 (n = 7)	31.8 ± 3.1	33.7 ± 3.1	37.0 ± 5.3	28.2 ± 2.7
Day 5 (n = 6)	33.7 ± 5.7	35.7 ± 5.5	36.5 ± 5.2	34.7 ± 1.3†

ND, Not done.
*$P < .05$ compared with days 1 and 3 in allografts by using Scheffe method.
†$P < .05$ compared with day 3 in isografts by using Scheffe method.

in mediastinal lymph nodes on day 5 was also significantly increased compared with that on days 3 and 1 in allografts (Table 3). In the mesenteric lymph nodes, spleen, and PBLs, there were no significant changes in the proportion of $CD4^+/CD45RC^-$ cells to lymphoid cells and in the proportion of $CD4^+/CD45RC^-$ cells to $CD4^+$ cells during the progress of the acute rejection after allograft transplantation. Thus the changes in lymphocyte subsets in the allografts were restricted to the regional lymph nodes.

In the isograft recipients neither the proportion of $CD4^+/CD45RC^-$ cells to lymphoid cells nor the proportion of $CD4^+/CD45RC^-$ cells to $CD4^+$ cells changed in mediastinal lymph nodes, mesenteric lymph nodes, and the spleen. Interestingly, the ratio of $CD4^+/CD45RC^-$ cells to lymphoid cells and the ratio of $CD4^+/CD45RC^-$ cells to $CD4^+$ cells increased in PBLs 5 days after lung transplantation compared with that after 3 days (Tables 2 and 3).

Increase of $CD4^+/CD45RC^-$ Cells in BALF After Allograft Transplantation

We then examined the $CD4^+/CD45RC^-$ expression profile in the BALF to determine whether the changes in lymphocyte subpopulations could be measured through lavage. Representative results are shown in Figure 4. The dot counts on days 1 and 3 are lower than that on day 5 because there were fewer lymphocytes in the BALF on days 1 and 3. Although the proportion of $CD4^+/CD45RC^-$ cells to lymphocytes in the BALF gradually increased with the advance of acute rejection in allografts, statistical analysis was not able to reach a significant difference (Figure 5, *A*). On day 1, about half the $CD4^+$ cells in the BALF were $CD45RC^-$ cells. The proportion of $CD4^+/CD45RC^-$ cells to $CD4^+$ cells significantly increased on days 3 and 5 compared with that on day 1, and almost all $CD4^+$ cells became $CD45RC^-$

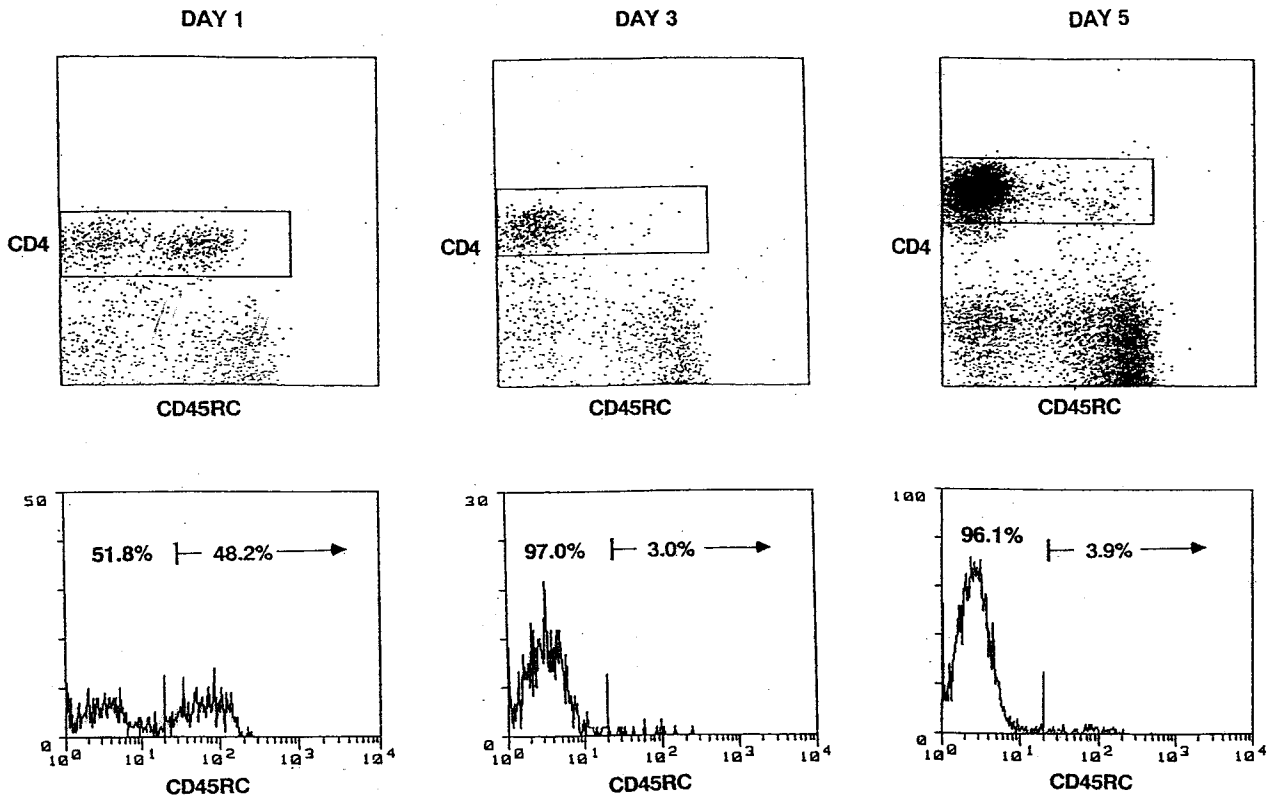

Figure 4. Representative results of the FACS analysis in the BALF recovered from the allografts. Two-dimensional dot-plotted graphs show the results of the 2-parameter analysis by CD4 and CD45RC for the lymphocytes in the BALF. Histograms show the results of the percentage of $CD4^+/CD45RC^-$ cells in total $CD4^+$ cells.

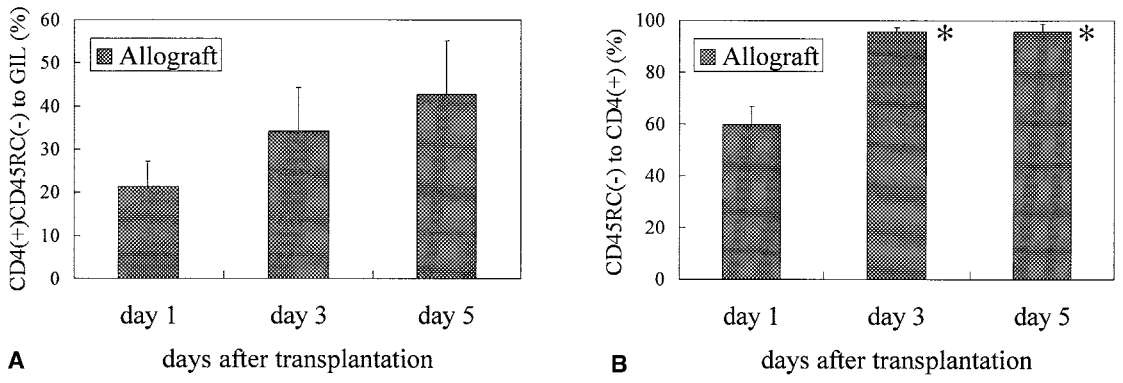

Figure 5. $CD4^+/CD45RC^-$ cells increased in the BALF collected from the allografted lungs. A, Proportion of $CD4^+/CD45RC^-$ cells was increased in allografts. B, Proportion of $CD4^+/CD45RC^-$ cells to $CD4^+$ cells significantly increased in the BALF recovered from the allografted lungs. On day 3, most of the $CD4^+$ cells in the BALF were $CD45RC^-$ cells. *$P < .01$ versus day 1 by using the Scheffe method.

cells (Figure 5, *B*). Almost all $CD4^+$ cells in the BALF were $CD45RC^-$ cells on days 3 and 5 in allografts. The ratio of $CD4^+/CD45RC^-$ cells to $CD4^+$ cells might be a very sensitive indicator of acute rejection of lung grafts.

Discussion

We have previously demonstrated that the maximal increase of GILs during the progress of acute rejection in lung allografts was on day 5 after transplantation,[3,4] and the

lymphocyte infiltration showed a latent phase on day 1, a vascular phase on day 3, and an alveolar phase on day 5 on the basis of morphologic criteria described by Prop and coworkers.[21] Similar results were seen in the present study (Figure 1). In this study we showed that the subpopulations of lymphocytes also significantly changed after allograft transplantation, with an increasing percentage of T cells and a decreasing percentage of B cells. The $CD4^+/CD8^+$ ratio also changed. Most interestingly, we found that the $CD4^+/CD45RC^-$ cells increased further compared with the total GILs. This increase appears mainly to be the result of the $CD4^+/CD45RC^-$ cells rather than the total $CD4^+$ cells on day 5 after transplantation. This change is specific for the grafts because a similar change was found only in the regional mediastinal lymph nodes and not in the mesenteric lymph nodes, spleen, and peripheral blood. Thus the acute lung graft rejection might be not only due to the increase in number of lymphocytes but also due to the shift of lymphocyte subpopulations.

Fournier and colleagues[22] investigated immunocyte expression in the respiratory tract in patients undergoing lung transplantation using antibodies against CD4, CD8, CD45RO, and HLADR. Immunohistologic findings indicated that the immunocyte profile in the respiratory tract did not reflect the process of acute rejection.[22] In that study the labeled immunocyte count was even less in the patients undergoing lung transplantation than that in nonsmoking control subjects. This seems to be attributable to the large number of patients who exhibited minor acute rejection rated as grade 1 because of the administration of immunosuppressants. Ibrahim and coworkers have previously shown an increased expression of CD45RO cells during human liver,[16] heart,[13] and kidney[23] allograft rejection. Thus the switch from $CD45^{high}$ to $CD45^{low}$ subtypes could be a common phenomenon in acute rejection of allografts.

Importantly, we found that the expression of $CD4^+/CD45RC^-$ cells in BALF was also increased. The proportion of $CD4^+/CD45RC^-$ cells to $CD4^+$ lymphocytes in BALF showed a significant increase from 60% on day 1 to about 96% on days 3 and 5 (Figure 5, *B*). The increase of $CD4^+/CD45RC^-$ cells to $CD4^+$ cells in the grafts was relatively smaller and late (from 57% on day 1 to 63% on day 3 and 86% on day 5; Figure 3, *B*). Increased expression of this subset of $CD4^+$ cells from BALF therefore might be an early marker to detect acute lung graft rejection. The existence of bronchus-associated lymphoid tissue in the central bronchus might contribute to the earlier increase in $CD4^+/CD45RC^-$ cells in the BALF. After lung transplantation, recipient lymphocytes might infiltrate to the bronchus-associated lymphoid tissue of the grafts in a relatively early phase, and recipient and donor lymphocytes might interact in these tissues.

The CD45R molecules are key molecules on lymphoid cell surfaces. The relationship between the expression of CD45RC on CD4 T cells and their functions are still not clear. $CD4^+/CD45RC^+$ T cells are associated with resting cells, whereas $CD4^+/CD45RC^-$ T cells are associated with cells activated by the contact with previous antigens. It has been shown that T cells stimulated in vitro lose $CD45R^{high}$ molecules and thus synthesize $CD45R^{low}$ molecules.[24-27] The increase of $CD4^+/CD45RC^-$ cells in the allografted lung might be a result of the stimulation of lymphocytes by alloantigens. On transplantation of skin allografts, $CD4^+/CD45RC^+$ T cells produce a larger amount of IL-2.[28] Others have found that $CD4^+/CD45RC^-$ T cells help B cells to produce a larger amount of IL-4.[29,30] Therefore $CD4^+/CD45RC^+$ cells and $CD4^+/CD45RC^-$ cells might correspond to T_H1-like and T_H2-like subsets, respectively.[31] However, the relationship between the CD45RC phenotype and T_H1-like/T_H2-like dichotomy is still unclear.[32,33] Interestingly, it has been shown that CD45RB mAb could prevent and reverse renal allograft rejection in mice[34] and could prolong xenograft survival in rat-to-mouse kidney and heart transplantation.[35] Thus the role of CD45R molecules in allograft rejection and tolerance is still controversial and merits further investigation.

Nevertheless, our results suggest that the increase in $CD4^+/CD45RC^-$ cells in allograft lung transplants might be a marker of acute rejection of lung grafts, which can be examined by means of bronchoalveolar lavage. However, because we did not use immunosuppressants in this animal model, the expression of this subtype of lymphocytes needs to be further studied in the clinical setting.

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